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**INBRED CORN LINE G1704**

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## **INBRED CORN LINE G1704**

### **FIELD OF THE INVENTION**

10 This invention is in the field of corn breeding, specifically relating to an inbred corn line designated G1704. This invention also is in the field of hybrid maize production employing the present inbred.

### **BACKGROUND OF THE INVENTION**

15 The original maize plant was indigenous to the Western Hemisphere. The plants were weedlike and only through the efforts of early breeders were cultivated crop species developed. The crop cultivated by early breeders, like the crop today, could be wind pollinated. The physical traits of maize are such that wind pollination results in self-pollination or cross-pollination between plants. Each plant has a separate male and female  
20 flower that contributes to pollination, the tassel and ear, respectively. Natural pollination occurs when wind transfers pollen from tassel to the silks on the corn ears. This type of pollination has contributed to the wide variation of maize varieties present in the Western Hemisphere.

25 The development of a planned breeding program for maize only occurred in the last century. A large part of the development of the maize product into a profitable agricultural crop was due to the work done by land grant colleges. Originally, maize was an open pollinated variety having heterogeneous genotypes. The maize farmer selected uniform ears from the yield of these genotypes and preserved them for planting the next season.  
30 The result was a field of maize plants that were segregating for a variety of traits. This type of maize selection led to, at most, incremental increases in seed yield.

Large increases in seed yield were due to the work done by land grant colleges that resulted in the development of numerous hybrid corn varieties in planned breeding

5 programs. Hybrids were developed by selecting corn lines and selfing these lines for several generations to develop homozygous pure inbred lines. One selected inbred line was crossed with another selected inbred line to produce hybrid progeny (F1). The resulting hybrids, due to heterosis, are robust and vigorous plants. Inbreds on the other hand are mostly homozygous. This homozygosity renders the inbred lines less vigorous.

10 Inbred seed can be difficult to produce since the inbreeding process in corn lines decreases the vigor. However, when two inbred lines are crossed, the hybrid plant evidences greatly increased vigor and seed yield compared to open pollinated, segregating maize plants. An important consequence of the homozygosity and the homogeneity of the inbred maize lines is that all hybrid seed produced from any cross of two such elite lines will

15 be the same hybrid seed and make the same hybrid plant. Thus the use of inbreds makes hybrid seed which can be reproduced readily. The hybrid plant in contrast does not produce hybrid seed that is readily reproducible. The seed on a hybrid plant is segregating for traits.

20 The ultimate objective of the commercial maize seed companies is to produce high yielding, agronomically sound plants that perform well in certain regions or areas of the Corn Belt. To produce these types of hybrids, the companies must develop inbreds, which carry needed traits into the hybrid combination. Hybrids are not often uniformly adapted for the entire Corn Belt, but most often are specifically adapted for regions of the Corn Belt.

25 Northern regions of the Corn Belt require shorter season hybrids than do southern regions of the Corn Belt. Hybrids that grow well in Colorado and Nebraska soils may not flourish in richer Illinois and Iowa soil. Thus, a variety of major agronomic traits are important in hybrid combination for the various Corn Belt regions, and have an impact on hybrid performance.

30 Inbred line development and hybrid testing have been emphasized in the past half-century in commercial maize production as a means to increase hybrid performance. Inbred development is usually done by pedigree selection. Pedigree selection can be

5 selection in an  $F_2$  population produced from a planned cross of two genotypes (often elite inbred lines), or selection of progeny of synthetic varieties, open pollinated, composite, or backcrossed populations. This type of selection is effective for highly inheritable traits, but other traits, for example, yield requires replicated test crosses at a variety of stages for accurate selection.

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Maize breeders select for a variety of traits in inbreds that impact hybrid performance along with selecting for acceptable parental traits. Such traits include: yield potential in hybrid combination; dry down; maturity; grain moisture at harvest; greensnap; resistance to root lodging; resistance to stalk lodging; grain quality; disease and insect  
15 resistance; ear and plant height. Additionally, Hybrid performance will differ in different soil types such as low levels of organic matter, clay, sand, black, high pH, low pH; or in different environments such as wet environments, drought environments, and no tillage conditions. These traits appear to be governed by a complex genetic system that makes selection and breeding of an inbred line extremely difficult. Even if an inbred in hybrid combination has  
20 excellent yield (a desired characteristic), it may not be useful because it fails to have acceptable parental traits such as seed yield, seed size, pollen production, good silks, plant height, etc.

To illustrate the difficulty of breeding and developing inbred lines, the following  
25 example is given. Two inbreds compared for similarity of 29 traits differed significantly for 18 traits between the two lines. If 18 simply inherited single gene traits were polymorphic with gene frequencies of 0.5 in the parental lines, and assuming independent segregation (as would essentially be the case if each trait resided on a different chromosome arm), then the specific combination of these traits as embodied in an inbred would only be expected to  
30 become fixed at a rate of one in 262,144 possible homozygous genetic combinations. Selection of the specific inbred combination is also influenced by the specific selection environment on many of these 18 traits which makes the probability of obtaining this one inbred even more remote. In addition, most traits in the corn genome are regrettably

5 not single dominant genes but are multi-genetic with additive gene action not dominant  
gene action. Thus, the general procedure of producing a non segregating  $F_1$  generation  
and self pollinating to produce a  $F_2$  generation that segregates for traits and selecting  
progeny with the visual traits desired does not easily lead to a useful inbred. Great care  
and breeder expertise must be used in selection of breeding material to continue to  
10 increase yield and the agronomics of inbreds and resultant commercial hybrids.

Certain regions of the Corn Belt have specific difficulties that other regions may not  
have. Thus the hybrids developed from the inbreds have to have traits that overcome or at  
least minimize these regional growing problems. Examples of these problems include in  
15 the eastern corn belt Gray Leaf Spot, in the north cool temperatures during seedling  
emergence, in the Nebraska region CLN (corn Lethal necrosis and in the west soil that has  
excessively high pH levels. The industry often targets inbreds that address these issues  
specifically forming niche products. However, the aim of most large seed producers is to  
provide a number of traits to each inbred so that the corresponding hybrid can be useful in a  
20 broader regions of the Corn Belt. The new biotechnology techniques such as  
Microsatellites, RFLPs, RAPDs and the like have provided breeders with additional tools to  
accomplish these goals.

## SUMMARY OF THE INVENTION

The present invention relates to an inbred corn line G1704. Specifically, this invention relates to plants and seeds of this line. Additionally, this relates to a method of producing from this inbred, hybrid seed corn and hybrid plants with seeds from such hybrid seed. More particularly, this invention relates to the unique combination of traits that combine in corn line G1704.

Generally then, broadly the present invention includes an inbred corn seed designated G1704. This seed produces a corn plant.

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The invention also includes the tissue culture of regenerable cells of G1704 wherein the cells of the tissue culture regenerates plants capable of expressing the genotype of G1704. The tissue culture is selected from the group consisting of leaves, pollen, embryos, roots, root tips, guard cells, ovule, seeds, anthers, silk, flowers, kernels, ears, cobs, husks and stalks, cells and protoplasts thereof. The corn plant regenerated from G1704 or any part thereof is included in the present invention. The present invention includes regenerated corn plants that are capable of expressing G1704's genotype, phenotype or mutants or variants thereof.

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The invention extends to hybrid seed produced by planting, in pollinating proximity which includes using preserved maize pollen as explained in U.S. Patent 5,596,838 to Greaves, seeds of corn inbred lines G1704 and another inbred line if preserved pollen is not used; cultivating corn plants resulting from said planting; preventing pollen production by the plants of one of the inbred lines if two are employed; allowing cross pollination to occur between said inbred lines; and harvesting seeds produced on plants of the selected inbred. The hybrid seed produced by hybrid combination of plants of inbred corn seed designated G1704 and plants of another inbred line are apart of the present invention. This inventions scope covers hybrid plants and the plant parts including the grain and pollen

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5 grown from this hybrid seed.

The invention further includes a method of hybrid F1 production. A first generation (F1) hybrid corn plant produced by the process of planting seeds of corn inbred line G1704; cultivating corn plants resulting from said planting; permitting pollen from another inbred  
10 line to cross pollinate inbred line G1704; harvesting seeds produced on plants of the inbred; and growing a harvested seed are part of the method of this invention.

Likewise included is a first generation (F1) hybrid corn plant produced by the process of planting seeds of corn inbred line G1704; cultivating corn plants resulting from  
15 said planting; permitting pollen from inbred line G1704 to cross pollinate another inbred line; harvesting seeds produced on plants of the inbred; and growing a plant from such a harvested seed.

The inbred corn line G1704 and at least one transgenic gene adapted to give  
20 G1704 additional and/or altered phenotypic traits are within the scope of the invention. Such transgenes are usually associated with regulatory elements (promoters, enhancers, terminators and the like). Presently, transgenes provide the invention with traits such as insect resistance, herbicide resistance, disease resistance increased or decreased starch or sugars or oils, increased or decreased life cycle or other altered trait.

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The present invention includes inbred corn line G1704 and at least one transgenic gene adapted to give G1704 modified starch traits. Furthermore this invention includes the inbred corn line G1704 and at least one mutant gene adapted to give modified starch, acid or oil traits. The present invention includes the inbred corn line G1704 and at least one  
30 transgenic gene selected from the group consisting of: bacillus thuringiensis, the bar or pat gene encoding Phosphinothricin acetyl Transferase, Gdha gene, EPSP synthase gene, low phytic acid producing gene, and zein. The inbred corn line G1704 and at least one transgenic gene useful as a selectable marker or a screenable marker are covered by

5 the present invention.

A tissue culture of the regenerable cells of hybrid plants produced with use of G1704 genetic material is covered by this invention. A tissue culture of the regenerable cells of the corn plant produced by the method described above are also included.

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## DEFINITIONS

In the description and examples, which follow, a number of terms are used. In order to provide a clear and consistent understanding of the specifications and claims, including  
15 the scope to be given such terms, the following definitions are provided.

### **BL MOIST**

The moisture percentage of the grain at black layer, i.e., when 50% of the plants per plot have reached physiological maturity.

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### **COLD GERM**

Cold Germ is a measurement of seed germination under cold soil conditions. Data is reported as percent of seed germinating.

25 **ECB**

European corn borer is a maize eating insect. ECB I is the first brood generation of European corn borers. ECB II is the second generation of European corn borers. ECB I is a rating of leaf damage. The ECB II (ECB2) rating is based upon tunneling. For all Entomology ratings, the higher number is best (1= little or no resistance, 9= highly  
30 resistant). The scale is slightly different for Ear Rating, which is taken on a 1 - 4 basis. This is a rating of corn borer feeding on the ear. A 1 represents feeding over the entire ear, while a 4 represents no observable feeding on the ear.



5 **EMERGE (EMG)**

The number of emerged plants per plot (planted at the same seedling rate) collected when plants have two fully developed leaves.

**GI**

10 This is a selection index that provides a single quantitative measure of the worth of a hybrid based on four traits. FI is a very similar index which weights yield less than GI. In GI yield is the primary trait contributing to index values. The GI value is calculated by combining stalk lodging, root lodging, yield and dropped ears according to the attached mathematical formula:

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$$GI = 100 + 0.5 (YLD) - 0.9(\%STALK LODGE) - 0.9(\%ROOT LODGE) - 2.7(\%DROPPED EAR)$$

**GLS**

Gray Leaf Spot (Cercospora Zeae) disease rating. This is rated on a 1-9 scale with a "1" being very susceptible, and a "9" being very resistant.\*

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**GW**

Gross' Wilt (Corynebacterium nebraskense). This is rated on a 1-9 scale with a "1" being very susceptible, and a "9" being very resistant.\*

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**HEATP10**

The number of Growing Degree Units (GDU's) or heat units required for an inbred line or hybrid to have approximately 10 percent of the plants shedding pollen. This trait is measured from the time of planting. Growing Degree Units are calculated by the Barger Method where the GDU's for a 24 hour period are:

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$$GDU = \frac{(\text{Max Temp } (^{\circ}\text{F}) + \text{Min Temp } (^{\circ}\text{F}))}{2} - 50$$

5

The highest maximum temperature used is 86°F and the lowest minimum temperature used is 50°F. For each inbred or hybrid it takes a certain number of GDU's to reach various stages of plant development.

10 **HEATBL**

The number of GDU's after planting when approximately 50 percent of the inbred or hybrid plants in a plot have grain that has reached physiological maturity (black layer).

**HEATPEEK**

15 The number of GDU's after planting of an inbred when approximately 50 percent of the plants show visible tassel extension.

**HEATP50 or HTP50**

20 The number of GDU's required for an inbred or hybrid to have approximately 50 percent of the plants shedding pollen. Growing Degree Units are calculated by the Barger Method as shown in the HEATP10 definition.

**HEATP90**

25 The number of GDU's accumulated from planting when the last 100 percent of plants in an inbred or hybrid are still shedding enough viable pollen for pollination to occur. Growing Degree Units are calculated by the Barger Method as shown in the HEATP10 definition.

**HEATS10**

30 The number of GDU's required for an inbred or hybrid to have approximately 10 percent of the plants with silk emergence of at least 0.5 inches. Growing Degree Units are calculated by the Barger Method as shown in the HEATP10 definition.

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### **HEATS50 or HTS50**

The number of GDU's required for an inbred or hybrid to have approximately 50 percent of the plants with silk emergence of at least 0.5 inches. Growing Degree Units are calculated by the Barger Method as shown in the HEATP10 definition.

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### **HEATS90**

The number of GDU's required for an inbred or hybrid to have approximately 90 percent of the plants with silk emergence of at least 0.5 inches. Growing Degree Units are calculated by the Barger Method as shown in the HEATP10 definition.

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### **MDMV<sub>A</sub>**

Maize Dwarf Mosaic Virus strain A. The corn is rated on a 1-9 scale with a "1" being very susceptible, and a "9" being very resistant.\*

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### **MDMV<sub>B</sub>**

Maize Dwarf Mosaic Virus strain B. This is rated on a 1-9 scale with a "1" being very susceptible and a "9" being very resistant.\*

### **MOISTURE**

25 The average percentage grain moisture of an inbred or hybrid at harvest time.

### **NLB**

Northern Leaf Blight (Exserohilum turcicum) disease rating. This is rated on a 1-9 scale with a "1" being very susceptible, and a "9" being very resistant.\*

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### **PCT TILLER or TILLER RATING**

The total number of tillers per plot divided by the total number of plants per plot.

**PLANT**

This term includes the entire plant and its plant cells, plant protoplasts made from its cells, plant cell tissue cultures from which corn plants can be regenerated, plant calli, plant clumps, and plant cells that are intact in plants or parts of plants, such as embryos, pollen, flowers, kernels, ears, cobs, leaves, husks, stalks, roots, root tips, anthers, silk and the like, and this term also includes any mutated gene, transgenic DNA or (RNA) or portion thereof that have been introduced into the plant by whatever method.

**PLANT HEIGHT (PLTHT) (PHT)**

The distance in centimeters from ground level to the base of the tassel peduncle.

**PLANT INTEGRITY (PLTINT) or (INT)**

The level of plant integrity on a scale of 1-9 with 9 evidencing the trait most strongly: 1-2.9 ratings are low plant integrity, 3-5.9 ratings are intermediate plant integrity, and 6-9 ratings are strongly evidencing plant integrity.

**POPULATION (POP)**

The plant population.

**RM**

Predicted relative maturity based on the moisture percentage of the grain at harvest. This rating is based on known set of checks and utilizes standard linear regression analyses and is referred to as the Minnesota Relative Maturity Rating System.

**SHED**

The volume of pollen shed by the male flower rated on a 1-5 scale where a "1" is a very light pollen shedder, a "2.5" is a moderate shedder, and a "5" is a very heavy shedder.

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**SLB**

Southern Leaf Blight (Bipolaris maydis) disease rating. This is rated on a 1-9 scale with a "1" being very susceptible, and a "9" being very resistant.\*

10 **STAYGREEN (SGN)**

The level of staygreen of the plant on a scale of 1-9 with 9 evidencing the trait most strongly: 1-2.9 ratings are low staygreen, 3-5.9 ratings are intermediate staygreen, and 6-9 ratings are strongly evidencing staygreen.

15 **TWT**

The measure of the weight of grain in pounds for a one bushel volume adjusted for percent grain moisture.

**VIGOR (VIG)**

20 Visual rating of 1 to 9 made 2-3 weeks post-emergence where a "1" indicates very poor early plant development, and a "9" indicates superior plant development.

**WARM GERM**

25 A measurement of seed germination under ideal (warm, moist) conditions. Data is reported as percent of seeds germinating.

**YIELD (YLD)**

Actual yield of grain at harvest adjusted to 15.5% moisture. Measurements are reported in bushels per acre.

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**% DROPPED EARS (DE)**

The number of plants per plot, which dropped their primary ear, divided by the total number

5 of plants per plot.

**% ROOT LODGE (RL)**

Percentage of plants per plot leaning more than 30 degrees from vertical divided by total plants per plot.

10 **% STALK LODGE (SL)**

Percentage of plants per plot with the stalk broken below the primary ear node divided by the total plants per plot.

**% Cull**

Percentage of seed that passes through a 16/64 inch screen or will not pass through a  
15 25/64 inch screen.

\*Resistant - on a scale of 1-9 with 9 evidencing the trait most strongly: 1-2.9 ratings are susceptible, 3-5.9 ratings are intermediate, and 6-9 ratings are resistant.

20 **DETAILED DESCRIPTION OF THE INVENTION**

G1704 has outstanding vigor for an inbred. The inbred tolerates heat well. This inbred fills its ear even in high heat summers. G1704 shows acceptable germination and does better when the early season conditions are not extremely wet. The inbred carries  
25 some disease tolerance.

The inbred has shown uniformity and stability within the limits of environmental influence for all the traits as described in the Variety Description Information (Table 1) that follows.

30

The inbred has been self-pollinated for a sufficient number of generations to give inbred uniformity. During plant selection in each generation, the uniformity of plant type was selected to ensure homozygosity and phenotypic stability. The line has been

5 increased in isolated farmland environments with data on uniformity and agronomic traits being observed to assure uniformity and stability. No variant traits have been observed or are expected in G1704.

10 The best method of producing the invention, G1704 which is substantially homozygous, is by planting the seed of G1704 which is substantially homozygous and self-pollinating or sib pollinating the resultant plant in an isolated environment, and harvesting the resultant seed.

**TABLE 1**

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**G1704**

**VARIETY DESCRIPTION INFORMATION**

20

#1 Type: Dent

#2 Region Best Adapted: Broadly adapted – in Southern Iowa and Northern Missouri and within that east-west band of the Corn Belt. This inbred usually has a RM of about 106-108 days. When in hybrid combination the inbred usually has RM of about 108-115 days.

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#3 Plant Traits

Plant Height 67 in.

Ear Height 28 in.

Tillers (Rating) 5

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Leaf Color Medium Green

Brace Root Color Green

Silk Color Green/Yellow

5   **#4   Tassel Traits**

Glume Ring Color    GREENISH

Anther Color        YELLOW

**#5   Ear and Kernel Traits**

Cob Color            Pink/Light Red

10        Kernel Crown Color YELLOW

Kernel Body Color   YELLOW

**#6   Disease Resistance In Inbred \***

15                                Gross' Wilt = 5.8

Northern Leaf Blight = 5.7

Eye Spot= 4.8

Gray Leaf Spot= 6.8

\* rating based on a 9-1 scale with a nine rating indicating most resistance and 1most susceptibility

20   **#7   Insect Resistance In Inbred**

ECB1 = 3.7

ECB2 = 5.8

Ear rate = 2.6

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The comparable inbred to G1704 is ZS01101 plus an additional trait of herbicide tolerance, specifically IT<sup>®</sup> (IT<sup>®</sup> is Advanta's trademark for imidazolinone tolerance). ZS01101 is described in U.S. patent 5,723,725. ZS01101/IT has a number of similarities to the present invention.

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The data provided above is often a color. The Munsell code is a reference book of color, which is known and used in the industry and by persons with ordinary skill in the art of plant breeding.



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The purity and homozygosity of inbred G1704 is constantly being tracked using isozyme genotypes as shown in Table 2.

### **Isozyme Genotypes for G1704**

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Isozyme data were generated for inbred corn line G1704 according to procedures known and published in the art. The data in Table 2 gives the electrophoresis data on G1704.

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**TABLE 2**  
**ELECTROPHORESIS RESULTS FOR G1704**

| <u>Patent Code</u> | <u>ACP1</u> | <u>ACP4</u> | <u>ADH</u> | <u>MDH1</u> | <u>MDH2</u> | <u>PGD1</u> | <u>PGD2</u> | <u>PHI</u> | <u>PGM</u> | <u>IDH2</u> |
|--------------------|-------------|-------------|------------|-------------|-------------|-------------|-------------|------------|------------|-------------|
| G1704              | 33          | 0           | 22         | 22          | 11          | 11          | 11          | 22         | 22         | 22          |
| G6103              | 33          | 55          | 22         | 22          | 22          | 11          | 11          | 22         | 22         | 22          |
| ZS01101/IT         | 11          | 0           | 22         | 22          | 11          | 11          | 11          | 22         | 22         | 22          |

### **Inbred and Hybrid Performance of G1704**

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The traits and characteristics of inbred corn line G1704 are listed to compare with other inbreds and/or in hybrid combinations. The G1704 data shows the characteristics and traits of importance, giving a snapshot of G1704 in these specific environments.

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Table 3A shows a comparison between G1704 and a comparable inbred ZS01101/IT. G1704 has significantly higher yield at harvest and less moisture than does ZS01101/IT. The two inbreds show significant differences in plant height. The two inbreds differ significantly across all Heat measurements for both pollen shed and silking. Both inbreds have excellent warm germination.

**TABLE 3A**  
**PAIRED INBRED COMPARISON DATA**

| <u>Inbreds</u> | <u>Yield</u> | <u>Moisture</u> | <u>Ear Height</u> | <u>Plant Height</u> | <u>Emerge</u> | <u>Heat Peek</u> |
|----------------|--------------|-----------------|-------------------|---------------------|---------------|------------------|
| G1704          | 101.0        | 11.2            | 73.1              | 172.2               | 89.5          | 1384.1           |
| ZS01101/IT     | 93.0         | 11.5            | 72.0              | 161.0               | 88.2          | 1400.4           |
| # Expts        | 28.0         | 28.0            | 13.0              | 13.0                | 28.0          | 23.0             |
| Diff           | 8.1          | 0.4             | 1.1               | 11.2                | 1.3           | 16.3             |
| Prob           | 0.003***     | 0.2             | 0.6               | 0.003***            | 0.2           | 0.035**          |

| <u>Inbreds</u> | <u>Heatp10</u> | <u>Heatp10</u> | <u>Heatp50</u> | <u>Heatp90</u> | <u>Heats10</u> | <u>Heats50</u> | <u>Heats90</u> |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| G1704          | 1426.9         | 1426.9         | 1459.1         | 1635.2         | 1432.6         | 1468.4         | 1501.3         |
| ZS0110         |                |                |                |                |                |                |                |
| 1/IT           | 1475.5         | 1475.5         | 1512.2         | 1690.5         | 1488.9         | 1531.1         | 1577.8         |
| # Expts        | 24.0           | 24.0           | 24.0           | 12.0           | 24.0           | 24.0           | 12.0           |
| Diff           | 48.6           | 48.6           | 53.1           | 55.3           | 56.3           | 62.8           | 76.4           |
| Prob           | 0.000***       | 0.000***       | 0.000***       | 0.000***       | 0.000***       | 0.000***       | 0.000***       |

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| <u>Inbreds</u> | <u>%Lrg Med Flat</u> | <u>%Lrg Med Rnd</u> | <u>%Lrg Plateless</u> | <u>%Sml Med Flat</u> | <u>%Sml Med Rnd</u> |
|----------------|----------------------|---------------------|-----------------------|----------------------|---------------------|
| G1704          | 40.4                 | 15.2                | 29.8                  | 4.8                  | 5.7                 |
| ZS01101/IT     | 5.4                  | 3.8                 | 0.1                   | 27.2                 | 13.7                |
| # Expts        | 28.0                 | 28.0                | 28.0                  | 28.0                 | 28.0                |
| Diff           | 35.0                 | 11.4                | 29.6                  | 22.4                 | 8.0                 |
| Prob           | 0.000***             | 0.000***            | 0.000***              | 0.000***             | 0.000***            |

| <u>Inbreds</u> | <u>%Sml Plateless</u> | <u>%Cull</u> | <u>Shed</u> | <u>Cold Germ</u> | <u>Warm Germ</u> |
|----------------|-----------------------|--------------|-------------|------------------|------------------|
| G1704          | 1.5                   | 2.5          | 3.3         | 90.1             | 97.4             |
| ZS01101/IT     | 28.5                  | 21.1         | 4.3         | 93.0             | 97.2             |
| # Expts        | 28.0                  | 28.0         | 24.0        | 11.0             | 10.0             |
| Diff           | 26.9                  | 18.6         | 1.0         | 3.0              | 0.2              |
| Prob           | 0.000***              | 0.000***     | 0.000***    | 0.2              | 0.7              |

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**TABLE 3B**

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## PAIRED HYBRID COMPARISON DATA

| <u>Hybrid</u> | <u>Yield</u> | <u>Fl</u> | <u>Gl</u> | <u>Y M</u> | <u>Moisture</u> | <u>%Root<br/>Lodge</u> | <u>%Stalk<br/>Lodge</u> | <u>%Root<br/>Lodge</u> | <u>Test<br/>Weight</u> |
|---------------|--------------|-----------|-----------|------------|-----------------|------------------------|-------------------------|------------------------|------------------------|
| CI/G1704      | 182.8        | 143.7     | 187.3     | 9.9        | 19.0            | 1.1                    | 3.3                     | 0                      | 56.9                   |
| CI/ZS01101    |              |           |           |            |                 |                        |                         |                        |                        |
| GLS/IT        | 174.4        | 135.1     | 183.2     | 8.7        | 20.9            | 1.4                    | 3.2                     | 0                      | 55.9                   |
| # Expts       | 122.0        | 120.0     | 120.0     | 122.0      | 122.0           | 121.0                  | 120.0                   | 120                    | 119                    |
| Diff          | 8.4          | 8.6       | 4.1       | 1.3        | 1.9             | 0.2                    | 0.2                     | 0                      | 1                      |
| Prob          | 0.000***     | 0.000***  | 0.000***  | 0.000***   | 0.000***        | 0.5                    | 0.8                     | 0.368                  | 0.000***               |

\*CI= common inbred in each hybrid

IT= Imidazolinone herbicide resistance by mutation

10 GLS= gray leaf spot trait

Table 3B shows the inbred G1704 in hybrid combination in comparison with another hybrid combination, which has a common inbred with the hybrid containing G1704. When in this hybrid combination the present inbred G1704 carries a significantly less moisture and a  
 15 distinctively significant increase in yield into the hybrid. The Y/M for the hybrid combination containing the present invention is significantly different to the compared hybrid.

### TABLE 3B1

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## PAIRED HYBRID COMPARISON DATA

| <u>Hybrid</u> | <u>Yield</u> | <u>Fl</u> | <u>Gl</u> | <u>Y M</u> | <u>Moisture</u> | <u>%Root<br/>Lodge</u> | <u>%Stalk<br/>Lodge</u> | <u>%Root<br/>Lodge</u> | <u>Test<br/>Weight</u> |
|---------------|--------------|-----------|-----------|------------|-----------------|------------------------|-------------------------|------------------------|------------------------|
| CI/G1704      | 185.4        | 144.2     | 188.7     | 9.7        | 19.4            | 1.6                    | 2.5                     | 0.0                    | 56.8                   |
| 8348*         | 186.8        | 137.1     | 188.7     | 8.6        | 22.5            | 2.3                    | 2.6                     | 0.1                    | 55.7                   |
| # Expts       | 166.0        | 164.0     | 164.0     | 166.0      | 166.0           | 166.0                  | 164.0                   | 165.0                  | 158.0                  |
| Diff          | 1.4          | 7.1       | 0.0       | 1.1        | 3.1             | 0.7                    | 0.1                     | 0.1                    | 1.1                    |
| Prob          | 0.5          | 0.000***  | 1.0       | 0.000***   | 0.000***        | 0.2                    | 0.5                     | 0.010***               | 0.000***               |

25 \*CI= common inbred in each hybrid

5 Table 3B1 shows the inbred G1704 in hybrid combination in comparison with another hybrid combination, which has ainbred in common with the hybrid containing G1704. When in this hybrid combination the present inbred G1704 carries in to the hybrid a significantly less moisture and similar yield. The Y/M for the hybrid combination containing the present invention is significantly different to the compared hybrid. The  
10 present inbred in hybrid combination is also significantly different root lodging and test weight.

Table 4 shows the GCA (general combining ability) estimates of G1704 compared with the GCA estimates of the other inbreds. The estimates show the general combining ability is weighted by the number of experiment/location combinations in which the specific  
15 hybrid combination occurs. The interpretation of the data for all traits is that a positive comparison is a practical advantage. A negative comparison is a practical disadvantage. The general combining ability of an inbred is clearly evidenced by the results of the general combining ability estimates. This data compares the inbred parent in a number of hybrid combinations to a group of "checks". The check data is from other companies' hybrids,  
20 particularly the leader in the industry and Garst Seed's commercial products and pre-commercial hybrids, which were grown in the same sets and locations. This Table shows that 32 different crosses were analyzed. Each of these crosses were made in small plots with not less than 50 hybrid seeds being planted per cross. These experiments produced progeny of G1704 by a number of different lines. The grain produced by these hybrids  
25 have the present invention as an ancestor. The hybrid grain would be genetically different from the hybrid seed that formed the plant on which the hybrid grain was produced. However, such grain would be readily identifiable as being progeny of the invention or as having the invention as an ancestor.

TABLE 4

## G1704

One parent in  
each hybrid  
tested to  
provide this  
data is G1704

|     | <u>N in</u> |           | <u>N in</u> | <u>Total</u> |          |           |          |          |           |          |            |            |            |            |            |            |            |           |
|-----|-------------|-----------|-------------|--------------|----------|-----------|----------|----------|-----------|----------|------------|------------|------------|------------|------------|------------|------------|-----------|
|     | <u>YR</u>   | <u>YR</u> | <u>YR</u>   | <u>YR</u>    | <u>N</u> | <u>FI</u> | <u>Y</u> | <u>M</u> | <u>GI</u> | <u>I</u> | <u>YLD</u> | <u>MST</u> | <u>%SL</u> | <u>%RL</u> | <u>%DE</u> | <u>TWT</u> | <u>POP</u> | <u>RM</u> |
|     | <u>1</u>    | <u>2</u>  | <u>3</u>    | <u>4</u>     |          |           |          |          |           |          |            |            |            |            |            |            |            |           |
| XR= | 54          | 140       | 236         | 444          | 0.5      | 0.2       | -0.8     | 0.1      |           |          | 0.4        | 0.6        | -0.8       | -0.3       | 0          | 0.8        | -21        | 111       |
| XH= | 54          | 140       | 236         | 31           | -1.3     | 0.2       | -2.9     | 1.4      |           |          | -2.6       | 0.7        | -1.1       | -0.8       | 0          | 1.3        | -138       | 112       |
| XT= | 54          | 140       | 236         | 9            | -0.1     | 0.2       | -0.9     | 0.4      |           |          | 1.7        | 0.3        | -1.1       | -0.7       | 0          | 0.8        | 72         | 112       |

XR=GCA Estimate:Weighted by Experiment

XH=GCA Estimate:Weighted by Parent2

XT=Same as XH but using only those parent2 with two years of data

FI=  $100 + 0.5 (Yld) - 2.3(MST) - 0.9(\%SL) - 0.9(\%RL) - 2.7(\%DE)$

POP=plants per acre

RM=The Minnesota Relative Maturity

Table 4 shows G1704 in XR crossed to 32 different inbreds to form hybrid combinations. G1704 in hybrid combination in XR and XH and XT shows an advantage for Y/M, moisture and an advantage for test weight. In XT and XR G1704 in hybrid combination shows a positive advantage for yield when compared to the commercial checks and the company's other inbreds in hybrid combination.

TABLE 5A

## YIELD RESPONSE

## Research Plots

| <u>HYBRID</u>    |              | <u>YIELD</u>             |           |            |            |            |            |            |  |
|------------------|--------------|--------------------------|-----------|------------|------------|------------|------------|------------|--|
|                  |              | <u>Environment Yield</u> |           |            |            |            |            |            |  |
| <u>Hybrid</u>    | <u>Error</u> | <u># Plots</u>           | <u>75</u> | <u>100</u> | <u>125</u> | <u>150</u> | <u>175</u> | <u>200</u> |  |
| CI/G1704         | 10.9         | 55                       | 84        | 108        | 132        | 156        | 180        | 204        |  |
| CI/ZS01101GLS/IT | 14           | 10281                    | 87        | 109        | 131        | 153        | 174        | 196        |  |
| CI/G1704         | 10.9         | 55                       | 84        | 108        | 132        | 156        | 180        | 204        |  |
| 8348             | 17           | 1868                     | 70        | 98         | 125        | 152        | 179        | 206        |  |

**TABLE 5B**  
**YIELD RESPONSE**

**STRIP TESTS**

| <u>HYBRID</u>        |              |                 | <u>YIELD</u>             |     |     |     |     |     |
|----------------------|--------------|-----------------|--------------------------|-----|-----|-----|-----|-----|
| <u>Hybrid</u>        | <u>Error</u> | <u># Strips</u> | <u>Environment Yield</u> |     |     |     |     |     |
|                      |              |                 | 75                       | 100 | 125 | 150 | 175 | 200 |
| CI/G1704             | 10.5         | 211             | 83                       | 107 | 131 | 154 | 178 | 202 |
| CI/ZS01101GLS<br>/IT | 11           | 1837            | 77                       | 102 | 126 | 151 | 175 | 200 |
| CI/G1704             | 10.5         | 211             | 83                       | 107 | 131 | 154 | 178 | 202 |
| 8348                 | 13.5         | 994             | 70                       | 97  | 124 | 150 | 177 | 204 |

- 10 Table 5A shows the research plot yield response of G1704 in hybrid combination in comparison with the plants in the environment around it at the same location and in comparison to two other hybrids. The data for the present inbred is showing consistently higher results in comparison to the environment level. G1704 in hybrid combination, is a
- 15 yields that exceed the environment yields regardless of yield potential of that environment. Table 5B shows the data from strip tests for the hybrid with the present inbred and two comparison hybrids. These comparison hybrids are not yielding higher in any environment except 8348 shows a higher yield in the highest yielding environment.

**TABLE 6A**  
**Agronomic Traits**

CI/G1704 VS  
CI/ZS01101GLS/IT

| <u>YEAR</u> | <u>#</u> | <u>Early Stand</u> | <u>Adv</u> | <u>Emg</u> | <u>Adv</u> | <u>Vigor</u> | <u>Adv</u> | <u>S50</u> | <u>Adv</u> | <u>P50</u> | <u>Adv</u> |
|-------------|----------|--------------------|------------|------------|------------|--------------|------------|------------|------------|------------|------------|
| 2001        | 6        | 95.8               | -3.3       | 6.0        | -0.6       | 6.3          | -0.6       |            |            |            |            |
|             | 6        | 99.2               |            | 6.6        |            | 6.9          |            |            |            |            |            |
| 2002        | 13       | 87.5               | -1.6       | 6.2        | 0.3        | 6.6          | 0.9        | 74.0       | -1.0       | 74.0       | 0.0        |
|             | 13       | 89.1               |            | 5.9        |            | 5.7          |            | 75.0       |            | 75.0       |            |
| 2003        | 16       | 92.0               | 0.6        | 6.4        | 0.1        | 5.9          | 1.0        | 77.0       | 0.0        | 77.0       | 0.0        |
|             | 16       | 91.3               |            | 6.2        |            | 4.9          |            | 77.0       |            | 77.0       |            |
| 2001-2003   | 35       | 90.4               | -0.6       | 6.2        | 0.1        | 6.3          | 0.7        | 76.0       | 0.0        | 76.0       | 0.0        |
|             | 35       | 91.0               |            | 6.2        |            | 5.6          |            | 76.0       |            | 76.0       |            |

5

| <u>Ear Ht.</u> | <u>Adv</u> | <u>Plant Ht.</u> | <u>Adv</u> | <u>Stay Green</u> | <u>Adv</u> | <u>Black Layer</u> | <u>Adv</u> | <u>Plant Int</u> | <u>Adv</u> |
|----------------|------------|------------------|------------|-------------------|------------|--------------------|------------|------------------|------------|
|                |            |                  |            | 7.0               | 0.0        |                    |            | 7.0              | -1.0       |
|                |            |                  |            | 7.0               |            |                    |            | 8.0              |            |
| 45.5           | 5.9        | 94.9             | 9.4        | 5.4               | -2.0       | 135.0              | -2.0       | 6.5              | -0.1       |
| 39.7           |            | 85.5             |            | 7.0               |            | 136.0              |            | 6.6              |            |
| 45.0           | 3.2        | 97.5             | 3.2        | 6.5               | -0.1       | 142.0              | 0.0        | 6.3              | -0.5       |
| 41.8           |            | 94.3             |            | 6.6               |            | 143.0              |            | 6.8              |            |
| 45.3           | 4.7        | 96.1             | 6.6        | 6.0               | -0.8       | 138.0              | -1.0       | 6.5              | -0.3       |
| 40.6           |            | 89.5             |            | 6.8               |            | 139.0              |            | 6.8              |            |

10 Lines marked with the advantage information have data from the present inbred in hybrid combination

- ADV= ADVANTAGE
- IT= Imidazolinone herbicide resistance

**TABLE 6A1**

**8424 vs 8348**

| <u>YEAR</u> | <u>#</u> | <u>Early Stand</u> | <u>Adv</u> | <u>Emg</u> | <u>Adv</u> | <u>Vigor</u> | <u>Adv</u> | <u>S50</u> | <u>Adv</u> | <u>P50</u> | <u>Adv</u> |
|-------------|----------|--------------------|------------|------------|------------|--------------|------------|------------|------------|------------|------------|
| 2002        | 13       | 87.5               | -0.9       | 6.2        | 0.3        | 6.6          | 0.3        | 74.0       | -3.0       | 74.0       | -2.0       |
|             | 13       | 88.4               |            | 5.9        |            | 6.3          |            | 77.0       |            | 76.0       |            |
| 2003        | 17       | 92.0               | 1.2        | 6.4        | -0.1       | 5.9          | 0.1        | 77.0       | -1.0       | 77.0       | -1.0       |
|             | 17       | 90.8               |            | 6.5        |            | 5.9          |            | 78.0       |            | 78.0       |            |
| 2002-2003   | 30       | 89.9               | 0.2        | 6.3        | 0.1        | 6.2          | 0.2        | 76.0       | -2.0       | 76.0       | -2.0       |
|             | 30       | 89.7               |            | 6.2        |            | 6.1          |            | 77.0       |            | 77.0       |            |

15

| <u>Ear Ht.</u> | <u>Adv</u> | <u>Plant Ht.</u> | <u>Adv</u> | <u>Stay Green</u> | <u>Adv</u> | <u>Black Layer</u> | <u>Adv</u> | <u>Plant Int</u> | <u>Adv</u> |
|----------------|------------|------------------|------------|-------------------|------------|--------------------|------------|------------------|------------|
| 45.5           | 0.7        | 94.9             | 4.8        | 5.4               | -0.9       | 135.0              | -5.0       | 6.5              | 0.1        |
| 44.8           |            | 90.1             |            | 6.3               |            | 140.0              |            | 6.4              |            |
| 45.2           | -0.7       | 96.9             | -1.8       | 6.5               | 0.4        | 142.0              | -2.0       | 6.3              | 1.3        |
| 45.9           |            | 98.7             |            | 6.1               |            | 144.0              |            | 5.0              |            |
| 45.3           | 0.0        | 95.9             | 1.5        | 5.9               | -0.3       | 138.0              | -3.0       | 6.4              | 0.5        |
| 45.4           |            | 94.4             |            | 6.2               |            | 142.0              |            | 5.9              |            |

5

The data in Table 6A and 6A1 shows the advantage or disadvantage associated with the agronomic traits of the present inbred when in hybrid combination when compared with a commercial. The Table also shows a commercial hybrid that has one inbred in common with the hybrid that includes the present invention. The last two lines of data show the combined data of all three years. The present invention when in hybrid combination in the overall data shows a positive advantage for emergence, and vigor in most years. The foregoing is set forth by way of example and is not intended to limit the scope of the invention.

10

15

This invention also is directed to methods for producing a corn plant by crossing a first parent corn plant with a second parent corn plant wherein the first or second parent corn plant is an inbred corn plant from the line G1704. Further, both first and second parent corn plants can come from the inbred corn line G1704 which produces a self of the inbred invention. The present invention can be employed in a variety of breeding methods which can be selected depending on the mode of reproduction, the trait, and the condition of the germplasm. Thus, any breeding methods using the inbred corn line G1704 are part of this invention: selfing, backcrosses, hybrid production, crosses to populations, haploid by such old and known methods of using stock material that induces haploids and anther culturing and the like.

20

25

All plants and plant cells produced using inbred corn line G1704 are within the scope of this invention. The invention encompasses the inbred corn line used in crosses with other, different, corn inbreds to produce (F1) corn hybrid seeds and hybrid plants and the grain produced on the hybrid plant. This invention includes plant and plant cells, which upon growth and differentiation produce corn plants having the physiological and morphological characteristics of the inbred line G1704.

30

Additionally, this maize can, within the scope of the invention, contain: a mutant gene



5 such as, but not limited to, the sugary 1 or shrunken 1 or waxy or AE or imazethapyr  
tolerant (IT or IR  $\text{TM}$ ) mutant gene; or transgenic genes such as but not limited to insect  
resistant genes such as Corn Rootworm gene, *Bacillus thuringiensis* (Cry genes), or  
herbicide resistant genes such as Pat gene or Bar gene, EPSP, or disease resistant genes  
such as the Mosaic virus resistant gene, etc., or trait altering genes such as flowering  
10 genes, oil modifying genes, senescence genes and the like. The methods and techniques  
for inserting, or producing and/or identifying a mutation or a transgene into the present  
invention through breeding, transformation, or mutating are well known and understood by  
those of ordinary skill in the art.

15 Various techniques for breeding and moving or altering genetic material within or  
into the present invention (whether it is an inbred or in hybrid combination) are also known  
to those skilled in the art. These techniques to list only a few are anther culturing, haploid  
production, (stock six is a method that has been in use for thirty years and is well known to  
those with skill in the art), transformation, irradiation to produce mutations, chemical or  
20 biological mutation agents and a host of other methods are within the scope of the  
invention. All parts of the G1704 plant including its plant cells produced using the inbred  
corn line are within the scope of this invention. The term transgenic plant refers to plants  
having genetic sequences, which are introduced into the genome of a plant by a  
transformation method and the progeny thereof. Transformation methods are means for  
25 integrating new genetic coding sequences into the plant's genome by the incorporation of  
these sequences into a plant through man's assistance, but not by breeding practices. The  
transgene once introduced into plant material and integrated stably can be moved into  
other germplasm by standard breeding practices.

30 Though there are a large number of known methods to transform plants, certain  
types of plants are more amenable to transformation than are others. Transformation of  
dicots is usually achievable for example, tobacco is a readily transformable plant. Monocots  
can present some transformation challenges, however, the basic steps of transforming

5 plants monocots have been known in the art for about 15 years. The most common method of maize transformation is referred to as gunning or microprojectile bombardment though other methods can be used. The process employs small gold-coated particles coated with DNA which are shot into the transformable material. Detailed techniques for gunning DNA into cells, tissue, callus, embryos, and the like are well known in the prior art.

10 One example of steps that can be involved in monocot transformation are concisely outlined in U.S. patent number 5,484,956 "Fertile Transgenic *Zea mays* Plants Comprising Heterologous DNA Encoding *Bacillus Thuringiensis* Endotoxin" issued January 16, 1996 and also in U.S. patent number 5,489,520 "Process of Producing Fertile *Zea mays* Plants and Progeny Comprising a Gene Encoding Phosphinothricin Acetyl Transferase" issued

15 February 6, 1996.

Plant cells such as maize can be transformed not only by the use of a gunning device but also by a number of different techniques. Some of these techniques include maize pollen transformation (See University of Toledo 1993 U.S. Patent No. 5,177,010); Whiskers technology (See U.S. patent numbers 5,464,765 and 5,302,523); electroporation;

20 PEG on Maize; *Agrobacterium* (See 1996 article on transformation of maize cells in Nature Biotechnology, Volume 14, June 1996) along with numerous other methods which may have slightly lower efficiency rates. Some of these methods require specific types of cells and other methods can be practiced on any number of cell types.

The use of pollen, cotyledons, zygotic embryos, meristems and ovum as the target

25 issue can eliminate the need for extensive tissue culture work. Generally, cells derived from meristematic tissue are useful. The method of transformation of meristematic cells of cereal is taught in the PCT application WO96/04392. Any number of various cell lines, tissues, calli and plant parts can and have been transformed by those having knowledge in the art. Methods of preparing callus or protoplasts from various plants are well known in

30 the art and specific methods are detailed in patents and references used by those skilled in the art. Cultures can be initiated from most of the above-identified tissue. The only true requirement of the transforming plant material is that it can form a transformed plant.

The DNA used for transformation of these plants clearly may be circular, linear, and double or single stranded. Usually, the DNA is in the form of a plasmid. The plasmid usually contains regulatory and/or targeting sequences which assists the expression of the gene in the plant. The methods of forming plasmids for transformation are known in the art.

10 Plasmid components can include such items as: leader sequences, transit polypeptides, promoters, terminators, genes, introns, marker genes, etc. The structures of the gene orientations can be sense, antisense, partial antisense, or partial sense: multiple gene copies can be used. The transgenic gene can come from various non-plant genes (such as; bacteria, yeast, animals, and viruses) along with being from plants.

15

The regulatory promoters employed can be constitutive such as CaMv35S (usually for dicots) and polyubiquitin for monocots or tissue specific promoters such as CAB promoters, MR7 described in U.S. patent 5,837,848, etc. The prior art promoters, includes but is not limited to, octopine synthase, nopaline synthase, CaMv19S, mannopine  
20 synthase. These regulatory sequences can be combined with introns, terminators, enhancers, leader sequences and the like in the material used for transformation.

The isolated DNA is then transformed into the plant. After the transformation of the plant material is complete, the next step is identifying the cells or material, which has been  
25 transformed. In some cases, a screenable marker is employed such as the beta-glucuronidase gene of the *uidA* locus of *E. coli*. Then, the transformed cells expressing the colored protein are selected. In many cases, a selectable marker identifies the transformed material. The putatively transformed material is exposed to a toxic agent at varying concentrations. The cells not transformed with the selectable marker, which provides  
30 resistance to this toxic agent, die. Cells or tissues containing the resistant selectable marker generally proliferate. It has been noted that although selectable markers protect the cells from some of the toxic affects of the herbicide or antibiotic, the cells may still be slightly affected by the toxic agent by having slower growth rates. If the transformed

5 material was cell lines then these lines are regenerated into plants. The cells' lines are treated to induce tissue differentiation. Methods of regeneration of cellular maize material are well known in the art.

10 A deposit of at least 2500 seeds of this invention will be maintained by Advanta USA, Inc., 2369 330th Street, Slater, Iowa 50244. Access to this deposit will be available during the pendency of this application to the Commissioner of Patents and Trademarks and persons determined by the Commissioner to be entitled thereto upon request. All restrictions on availability to the public of such material will be removed upon issuance of a granted patent of this application by depositing at least 2500 seeds of this invention at the  
15 American Type Culture Collection (ATCC), at 10801 University Boulevard, Manassas, VA 20110. The date of deposit was XXXX. The ATCC number of the deposit is XXXXX and on xx day of the XX month of XXXX year the deposit was found viable when tested. The deposit of at least 2500 seeds will be from the same inbred seed taken from the deposit maintained by Advanta USA, Inc. The ATCC deposit will be maintained in that  
20 depository, which is a public depository, for a period of 30 years, or 5 years after the last request, or for the effective life of the patent, whichever is longer, and will be replaced if it becomes nonviable during that period.

25 Additional public information on some ZS designations may be available from the PVP Office, a division of the US Government.

Accordingly, the present invention has been described with some degree of particularity directed to the preferred embodiment of the present invention. It should be appreciated, though, that the present invention is defined by the following claims construed  
30 in light of the prior art so that modifications or changes may be made to the preferred embodiment of the present invention without departing from the inventive concepts contained herein.